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Anticancer effect of herbal drug *Asparagus racemosus*

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Abstract:

Various physicochemical constants such as ash values, extractive values, loss on drying, crude fibre content, swelling index and foaming index were carried out. These values will help in confirming the identity and purity of the plant. Any significant deviation in the percentage of any parameters reported in this work may indicate adulteration or substitution in the drug.

Qualitative estimation of inorganic elements, quantitative estimation of heavy metals and pesticide residue were carried out and it showed only trace amount of heavy metals (within the limits) and absence of pesticide residue.

These pharmacognostical details observed from the present study might offer reliable clues for the correct identification of the leaves of this plant in crude as well as fragmentary form and also ensures its differentiation from its substitutes and adulterants. This is first report on the pharmacognostical standardization on the leavesof *Asparagus racemosus* Willd,

Introduction:

Plant and herbs used in the folk and traditional medicine have been accepted currently as one of the main source of chemoprevention drug discovery and development [(13)]. Around 60% of currently used anticancer agents are derived in one- way or another from natural sources, including plants, marine source and microorganisms.

Herbal medicines are plant derived materials or preparations, which contain raw or processed ingredient from one or more plants with therapeutic value and used as dietary supplements to fight or prevent common diseases in various systems of medicine such as Ayurveda, Unani and Siddha. Plant products have been used throughout human history for various purposes including medicine [(14)]. Herbs can be viewed as biosynthetic chemical laboratories, producing a number of chemical compounds. Herbal drugs

ranges from parts of plants to isolated purified active constituents. They may come from mostly leaves, roots, barks, seeds and flower. They are eaten, swallowed, drunk, inhaled or applied to the skin. Several secondary metabolites are produced by the higher plants as natural defense against disease and infections. The Indian system of medicine known as Ayurveda uses mainly plant based drug or formulation to treat various disease including cancer: and around. Approximately 877 small molecule drugs are introduced worldwide between 1981- 2002, 61% can be traced back to their origin in natural products. Recent surveys suggest that one in three persons use medicinal natural products daily and possibly one in two cancer patients use them as well.

Thin layer chromatography of n-Hexane, Chloroform, Ethyl acetate and Ethanol extracts were carried out and the results are tabulated in Table 14.

Table. 1 Thin layer chromatography studies

S.No	Extracts	Solvent system	No. Of Spots	R_f value
1.	n-Hexane	Ethyl acetate: Chloroform:Ethanol (5:3:2)	3	0.48 0.51 0.53
2.	Chloroform	Ethyl acetate: Chloroform:Ethanol (5:3:2)	4	0.36 0.39 0.46 0.49
3.	Ethyl acetate	Ethyl acetate: chloroform(6:4)	4	0.45 0.50 0.52 0.53
4.	Ethanol	Ethyl acetate: chloroform(6:4)	3	0.29 0.31 0.32

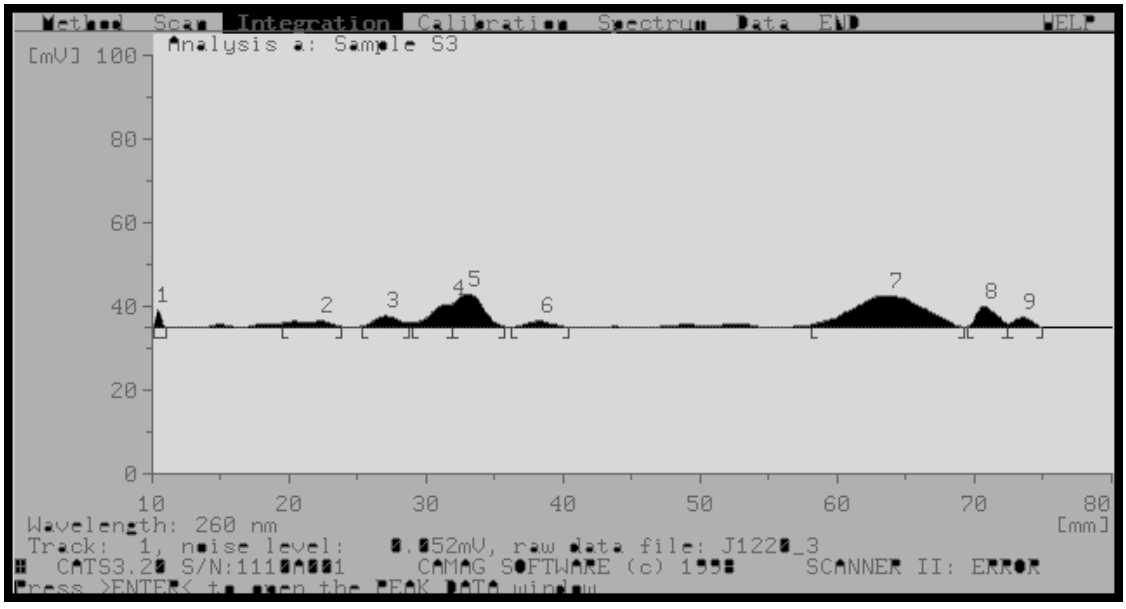
HPTLC

High performance thin layer chromatography (HPTLC) finger printing was performed with the ethyl acetate extract of *Asparagus racemosus* Willd, The chromatographic conditions were carried as detailed in material and method of this study. There were 9 peaks observed with different R_f values and different heights. Percentages of areas were also obtained from the chromatogram.

Table 2 HPTLC profile of ethyl acetate extracts

S.NO	Extract	Solvent system	mber ofspots	R_f values
		Toluene:Ethylacetate:Methanol:Formicacid (6:3:1:0.2)	9	11, 23, 28, 33, 34, 39, 64, 71, 74

Fig 3 Hptlc Finger Print For Ethyl Acetate Extract Of *Asparagus racemosus* Willd.,



In India most of the traditional knowledge on medical plants is in the oral form carried over generations to generations without any standard inventory. Necessary steps are needed for proper documentation, systematic regulation and widespread application. Since herbal medicines are prepared from materials of plant origin, they are prone to contamination, deterioration and variation in composition. Hence, before proceeding to clinical studies, scientists need to authenticate plants and also to detect their potency. A lot of analytical techniques have been developed for quality controlof drug from plant origin.

The quantitative phytochemical analysis for chloroform, ethyl acetate and ethanol extracts were performed. The total phenolic content was found to be 40.31±0.49, 60.85±0.29 &73.78±0.53µg/ml % w/w. The total tannin content was found

to be 14.73±0.37, 16.58±0.45&19.34±0.33µg/ml % w/w. The total flavanoid content was found to be 4.67±0.56, 7.98±0.48&36.44±0.62µg/ml % w/w.

***In vitro* Anticancer Activity**

Method: MTT Assay

Extracts used: Chloroform, ethyl acetate and ethanol extracts Cell line used

The human cervical cancer cell line (HeLa) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37^0C, 5% CO_2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly and the culture medium was changed twice a week.

Cell treatment procedure

The monolayer cells were binding with trypsin-ethylen diaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a heamocytometer and diluted with medium containing 5% FBS to give final density of $1x10^5$ cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^0C, 5% CO_2, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the test samples. They were initially dissolved in dimethylsulfoxide (DMSO) and an aliquot of the sample solution was diluted to twice

the desired final maximum test concentration with serum free medium. Additional four serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 h at 37^0C, 5% CO_2, 95% air and 100% relative humidity [16]. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

MTT assayPrinciple

This colorimetric asay is based on the capacity of a mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the 3- [4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) into a insoluble purple formazan product which is measured spectrophotometricallly. Only viable cellswith active mitochondria reduces the MTT, the amount of formazan produced is directly proportional to the number of viable cells.

Procedure

After 48 h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using 96 well plate counter. The % cell inhibition was determined using the following formula.

% Cell Inhibition = 100- Abs (sample)/Abs (control) x100.

Nonlinear regression graph was plotted between % Cell inhibition and Log concentration and IC_{50} was determined using GraphPad Prism software.

Results And Discussion

The *invitro* anticancer activity of the chloroform, ethyl acetate and ethanolextracts were given in Table 4,5.

Table 4. MTT Assay of Chloroform extract

Plant extract	Conc.µg/ml	Absorbance	% inhibition	IC_{50} µg/ml	R^2
	18.75	0.4053	2.1721		

Chloroform exrtract of *Asparagus racemosusus* Willd.,	**37.50**	**0.3633**	**12.3089**	**62.587µg/ml**	**0.9993**
	75	**0.1376**	**66.7739**		
	150	**0.2366**	**94.2880**		
	300	**0**	**100**		

Fig 4 Dose Response Curve Of Chloroform Extract Of

***Asparagus racemosus* for HELA cell line by MTT assay**

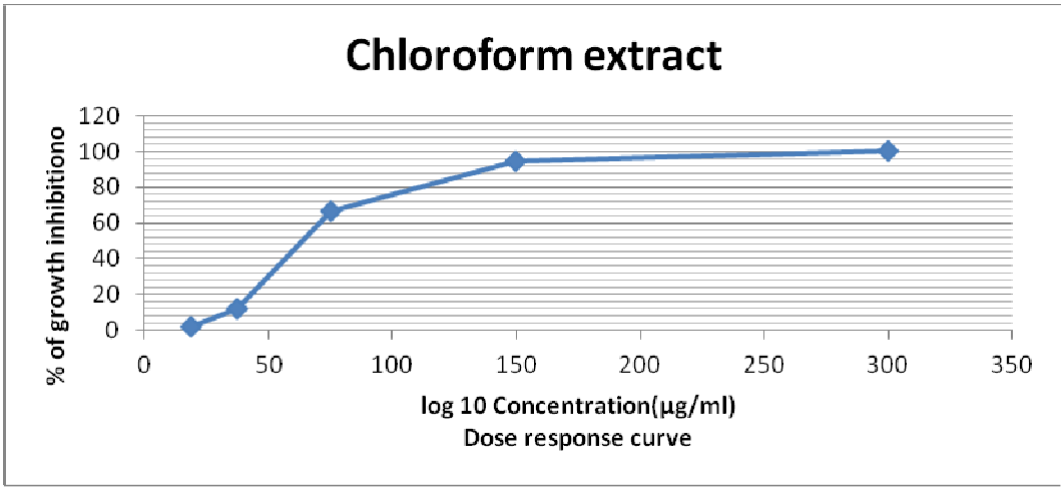


Table 5. MTT Assay of Ethyl acetate extract

Plant extract	Conc.µg/ml	Absorbance	% inhibition	IC_{50} µg/ml	R^2
Ethyl acetate exrtract of *Asparagus racemosusus* Willd.,	18.75	0.4133	0.2413	87.837µg/ml	0.9999
	37.50	0.4073	1.6894		
	75	0.2930	29.2839		
	150	0.0193	95.3338		
	300	0.0036	99.1150		

Fig 5 DOSE RESPONSE CURVE OF ETHYL ACETATE OF *Asparagusracemosus* FOR HELA CELL LINE BY MTT ASSAY

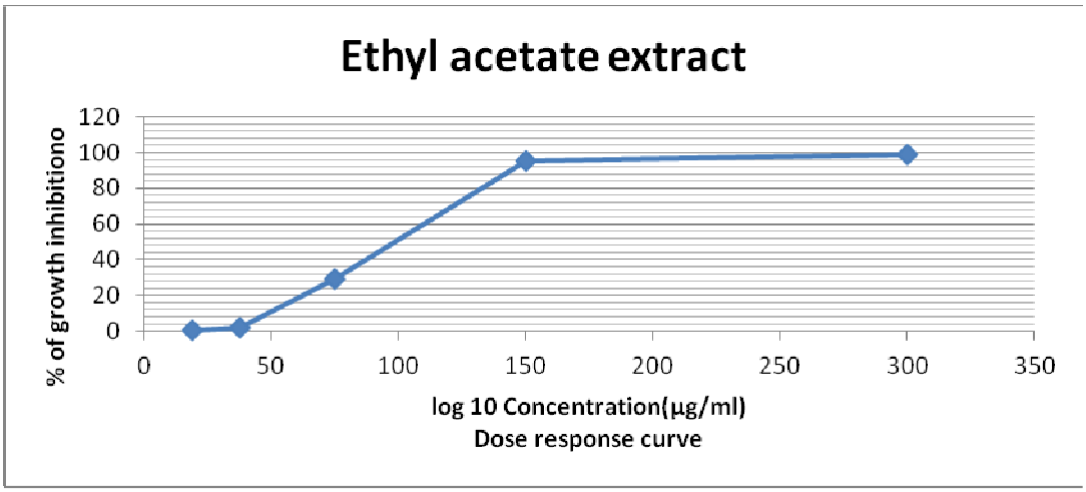
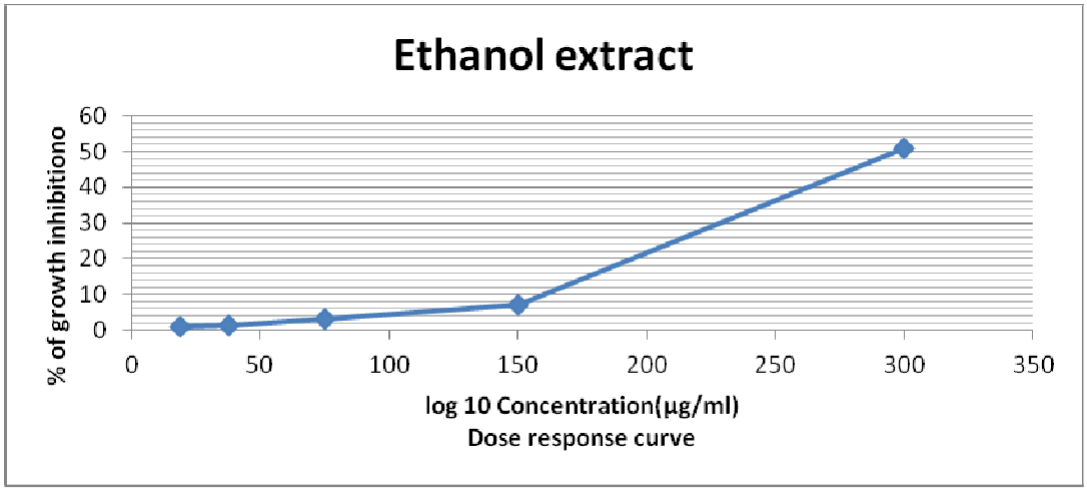


Table 6. MTT Assay of Ethanol extract

Plant extract	Conc.µg/ml	Absorbance	% inhibition	IC_{50} µg/ml	R^2
Ethanol extract of *Asparagus racemosus* Willd.,	18.75	0.4110	0.8045	297.7µg/ml	0.9953
	37.50	0.40833	1.4481		
	75	0.4016	3.0571		
	150	0.3836	7.1600		
	300	0.2040	50.764		

Fig 6 DOSE RESPONSE CURVE OF ETHANOL OF *Asparagus racemosus* FOR HELA CELL LINE BY MTT ASSAY



Discussion

The *in vitro* anticancer study for chloroform, ethyl acetate and ethanol extracts were carried out by MTT assay. The extracts were screened for its cytotoxicity against HeLa cell line at different concentration to determine the IC_{50} value.

The results are tabulated and graphically represented. The percentage growth inhibition was found to be increased with the increasing concentration of test compound. The IC_{50} value of Chloroform, Ethyl acetate and Ethanol extracts on the HeLa cell line were found to be 62.587, 87.837, 297.9 µg/ml and R^2 values were 0.9993, 0.9999, 0.9953 respectively. The chloroform and ethyl acetate extract showed significant *invitro* anticancer activity against HeLa cell line when compared to the ethanol which was taken for further anticancer studies.

The result suggests that the chloroform and ethyl acetate extract of *Asparagus racemosus* showed significant antitumor activity in EAC bearing mice. The effect of
A. racemosus extracts at the dose of 400mg/kg on biological parameters are discussedbelow.

Summary and Conclusion

Cancer is one of the leading causes of death worldwide. Currently available allopathic drugs for treating cancer causes number of side effects hence, people are now looking towards the herbal medicine. This paved a vital necessity for finding natural anticancer drug from herbal source. *Asparagus racemosus* Willd. is one such plant which is traditionally used in the treatment of cancer was selected for the present study.

The literature survey showed only scrappy information on the leaves of this plant. With this scanty of information on the leaves, consistent expectation of unexplored phytochemical profile and pharmacological efficacy forms the rationalefor the study.

Pharmacognostical studies

A perusal of literature showed paucity of pharmacognostical information on the leaves of this plant. Hence, it was carried out and reported for the first time.

In pharmacognostical studies macroscopy, microscopy, determination of physicochemical constants, analysis of inorganic elements, heavy metals and pesticide residue of the leaves were carried out.

Macroscopical study showed that the leaves are pine needle (phylloclades) shaped, sweet and bitter taste and dark green in colour. The various distinguishing features of leaves observed through anatomical studies were,

- Phyllodades 3-4 angled with lateral projections
- Radially oblong epidermal cells with prominent cuticle
- Circular chlorophyllous palisade cells
- Vascular strands with angular xylem and thick mass of phloemelements

The microscopical analysis of powder showed the presence of lignified fibres, epidermis with palisade cells and stomata.

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